Are foetal ultrasonographic and maternal blood progesterone measurements near parturition reliable predictors of the time of birth in the domestic cat?

Keiser, Ramona ; Reichler, Iris M ; Balogh, Orsolya

Abstract: In cats, accuracy of parturition day prediction by ultrasonographic measurement of foetal structures is decreasing towards the end of gestation. Foetal measurements during the last days of pregnancy are scarce. We determined foetal biparietal, abdominal and eye diameter (BPD, AD and ED, respectively) by ultrasonography as well as maternal blood progesterone (P4) within five days of delivery to predict parturition date and calculate accuracy of prediction. Foetal BPD at birth was compared with newborn kitten head diameter (HD). Kitten HD, crown-rump length (CRL) and body weight were compared by breed and gender. Ultrasonography measurements were carried out on the day of parturition in 14 queens, and on days 62–63 after the first mating and repeated 24–72 hr later in ten other cats. Accuracy of parturition day prediction using BPD and AD was determined based on the equations of Beccaglia et al. (2008) Veterinary Research Communications, 32(Suppl 1), S99 and Garcia Mitacek et al. (2015) Theriogenology, 84, 1131. Progesterone was measured at the time of presentation and repeated 24–72 hr later if parturition did not occur. Data were analysed with linear regression, t test, Mann–Whitney U test, one-way anova and Kruskal–Wallis test. There was a moderate relationship between BPD, days before birth (DBB) and litter size. AD and DBB had a low agreement, and ED was not associated with DBB. BPD at birth was significantly related to HD. The accuracy of parturition day prediction using BPD and AD was 27–53% and 17–35%, respectively. Kitten HD was associated with body weight, and both were inversely related to litter size. Newborn biometric measurements differed by breed but not by gender. Progesterone decreased towards parturition and reached 3.18 ± 1.68 ng/ml on the day of delivery. In conclusion, close to birth, the combination of foetal ultrasonography and maternal blood P4 rather than each as a sole predictor of parturition is recommended.

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Are fetal ultrasonographic and maternal blood progesterone measurements near parturition reliable predictors of the time of birth in the domestic cat?

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Contents

In cats, accuracy of parturition day prediction by ultrasonographic measurement of fetal structures is decreasing towards the end of gestation. Fetal measurements during the last days of pregnancy are scarce. We determined fetal biparietal, abdominal and eye diameter (BPD, AD and ED, respectively) by ultrasonography as well as maternal blood progesterone (P4) within five days of delivery to predict parturition date and calculate accuracy of prediction. Fetal BPD at birth was compared with newborn kitten head diameter (HD). Kitten HD, crown-rump length (CRL) and body weight were compared by breed and gender. Ultrasonography measurements were carried out on the day of parturition in 14 queens, and on day 62-63 after the first mating and repeated 24-72 hours later in ten other cats. Accuracy of parturition day prediction using BPD and AD was determined based on the equations of Beccaglia et al. (2008) and Garcia Mitacek et al. (2015). Progesterone was measured at the time of presentation and repeated 24-72 hours later if parturition did not occur. Data was analyzed with linear regression, T-test, Mann-Whitney U test, one-way ANOVA and Kruskal-Wallis test. There was a moderate relationship between BPD, days before birth (DBB) and litter size. AD and DBB had a low agreement, and ED was not associated with DBB. BPD at birth was significantly related to HD. The accuracy of parturition day prediction using BPD and AD was 27-53% and 17-35%, respectively. Kitten HD was associated with body weight, and both were inversely related to litter size. Newborn biometric measurements differed by breed but not by gender. Progesterone decreased towards parturition and reached 3.18 ± 1.68 ng/mL on the day of delivery. In conclusion, close to birth, the combination of fetal ultrasonography and maternal blood P4 rather than each as a sole predictor of parturition is recommended.

Keywords: prepartum, queen, ultrasound, parturition prediction, fetometry

Introduction

Ultrasonography is a suitable tool for pregnancy diagnosis in the queen, and allows estimation of gestational age or, alternatively, the date of delivery by measuring various fetal structures (Beccaglia et al., 2008; Zambelli & Prati, 2006). Prediction of parturition date is indicated if the day of mating is uncertain or inaccurately recorded. In these instances, the length of gestation is unknown or does not match the actual age of the pregnancy. In high risk pregnancies, e.g. previous dystocia, small litter size, history of pelvic fracture, advanced age of the queen, concurrent uterine disease and resorptions, intensive monitoring of the pregnancy with serial ultrasound examinations should be performed to ensure well-being and continuous development of the conceptuses. In the event when medical or surgical intervention may become necessary, e.g. in high risk pregnancies, knowledge about the actual length of gestation and thus the maturation status of the fetuses is crucial for optimal management. A full-length pregnancy in the domestic cat lasts 64-66 days and seems to be also influenced by breed and litter size (Garcia Mitacek et al., 2015; Musters, de Gier, Kooistra, & Okkens, 2011; Root Kustritz, 2006; Sparkes et al., 2006; Zambelli, Castagnetti, Belluzzi, & Paladini, 2004). Decreased viability of the kittens is expected if birth takes place before 60 days (Root Kustritz, 2006). Therefore, parturition date should always be estimated if an elective Cesarean section (C-section) is planned (Michel, Spörri, Ohlerth, & Reichler, 2011), and the surgery should be performed close to the expected date of delivery to ensure survival of the newborns.

In the second half of pregnancy, biparietal diameter (BPD), abdominal diameter (AD), gastric diameter and femur length of the fetuses showed the best correlation with gestational age or days before birth (DBB) (Beccaglia et al., 2008; Beck, Baldwin, & Bosu, 1990; Garcia Mitacek et al., 2015; Gatel et al., 2015; Zambelli, Castagnetti, Belluzzi, & Paladini, 2004;

Zambelli & Prati, 2006). Gestation length was positively associated with the queen's body weight, and negatively with the queen's age and litter size (Gatel et al., 2015). The equations derived from fitted regression curves, or the absolute values of the measurements at certain time points during pregnancy reported in these studies can be used to estimate delivery date in the clinical practice. Eye diameter (ED) has not yet been evaluated in cats, but in pony mares, a close relationship between eye diameter length (EDL) or width (EDW) with age of gestation in mid- to late-pregnancy was described (Turner et al., 2006). In that study, EDL was the best single predictor for days before parturition.

Unfortunately, the accuracy of parturition date prediction in the cat decreases as pregnancy advances (Beccaglia & Luvoni, 2012; Garcia Mitacek et al., 2015). Accuracy of estimated delivery date within one day using BPD dropped from 79% in weeks 5-6 of gestation, to 51% in week 9. Accuracy of prediction within two days remained above 80% until week 8, and declined to 70% in week 9 (Beccaglia & Luvoni, 2012). Nevertheless, in this study, dogs and cats were analyzed together. In the study of Garcia Mitacek et al. (2015), accuracy using BPD as well as AD measurements also decreased significantly from week 5 (80-95% and 50-90%) to week 9 (44-65% and 24-35%) of gestation, respectively. Furthermore, while Zambelli et al. (2004) reported BPD of 2.6 cm and AD of 3.83 cm 60 days after mating in eight domestic queens with a body weight of 3-3.5 kg, Garcia Mitacek et al. (2015) predicted BPD of 2.14 cm and AD of 3.16 cm for the same day of pregnancy in 16 mixed-breed colony cats with a body weight of 2-4 kg. Estimation of parturition day already seems to be difficult comparing cats of similar weight classes, and may be even more challenging in queens of different breed size and/or with different average kitten weight. For the clinician, it is important to know how well parturition date can be predicted close to birth in a less uniform population of cats, which is more representative of the patients of a veterinary practice. While the study of Gatel et al.

(2015) included a heterogeneous group of 24 queens, it had limited number of fetal measurements in the last three days before delivery.

Maternal blood progesterone (P4) may be used to estimate parturition date in the queen. On day 63-65 after mating, blood concentration of P4 was in general 4-5 ng/mL (Verhage, Beamer, & Brenner, 1976), 2.99 ± 1.29 ng/mL one day before (Garcia Mitacek et al., 2015), and on average 2.2 ng/mL on the day of parturition; in one queen however P4 above 10 ng/mL was detected (Schmidt, Chakraborty, & Wildt, 1983). Progesterone dropped to baseline levels immediately after delivery (Verhage, Beamer, & Brenner, 1976), although another study described higher than baseline concentrations for a variable time postpartum (Schmidt, Chakraborty, & Wildt, 1983).

The objective of our study was to find the best predictor of parturition date and to determine its accuracy by measuring fetal BPD, AD and ED ultrasonographically as well as P4 concentrations from maternal blood, on the day of delivery or maximum five days before parturition. Head diameter (HD) of the newborn kittens was measured postpartum as a control of BPD measured by ultrasonography on the day of birth. The relationships between HD and other biometric measurements, *i.e.*, crown-rump length (CRL) and body weight, were also analyzed.

Materials and methods

Animals

This prospective study was carried out on 24 pregnant domestic cats. The cats represented the following breeds: Domestic Shorthair (N=9), Bengal (N=6), Maine Coon (N=3), Birman (N=2), Norwegian Forest Cat (N=2), European Shorthair (N=1) and Egyptian Mau (N=1). Body weight and age of the queens was 4.5 ± 1.0 kg (mean ± SD; range: 3.1 – 7.4 kg) and 2.3

± 1.8 years (range: 0.8 – 8.5 years), respectively. Litter size was 3.7 ± 1.8 (range: 1 – 7). Three queens had singleton fetus. All owners were informed and signed a consent form. The study was approved by the Cantonal Veterinary Office of Zurich (permit no. ZH085/15).

The majority of the cats included in the study were presented at our clinic on the day of parturition due to dystocia (N=14), and underwent either emergency C-section (N=10) or conservative birth management (N=4). In two cases, only fetal or newborn kitten measurements were available. Seven additional animals were recruited by contacting cat breeders. These queens were examined 62-63 days after the first mating and re-examined every 48-72 hours until birth. Three of them had emergency C-section due to dystocia, and four cats gave birth naturally. Three further queens that were initially presented at our clinic for pregnancy evaluation in the second half of gestation were also recruited. They were examined within the scope of the study on day 62-63 after the first mating and re-examined every 48-72 hours until parturition. If mating date was unknown, day 62 was estimated based on the regression equation of Beccaglia et al. (2008). One queen underwent emergency C-section and another one conservative birth management due to dystocia. The third queen gave birth naturally.

Abdominal ultrasonography and measurement of fetal structures

Abdominal ultrasonography was performed with a LOGIQ e ultrasound machine (GE Healthcare, Little Chalfont, UK) using a linear 12 MHz probe. In all except three queens, BPD was measured in 2-4 fetuses of each cat. In the remaining three queens, who were presented because of dystocia, only one fetal BPD was obtained due to time constraint. Additionally, AD and ED were measured in 1-3 fetuses of 18 and 19 dams, respectively. BPD, AD and ED were obtained as previously described (Beccaglia & Luvoni, 2006; Turner et al., 2006; Zambelli,

Castagnetti, Belluzzi, & Paladini, 2004). BPD was measured at the widest distance of the fetal skull between the two parallel parietal bones, with the falx cerebri visible in the midline. AD was measured in a transverse image, at the level of the fetal stomach and liver. The vitreous body of the fetal eye was identified at the longest and widest aspect with the lens visible in the ultrasound image, and EDL and EDW were measured perpendicular to each other (Figure 1). The measurement line of EDW dissected the lens approximately in the middle at a 90º angle.

Accuracy of BPD and AD measurements

The accuracies of parturition day prediction by measuring fetal BPD and applying the linear regression equation of Beccaglia et al. (2008), *i.e.*, DBB = (BPD in mm – 23.39)/0.47 as well as that of Garcia Mitacek et al. (2015), *i.e.*, DBB = (BPD in mm - 23.0844)/0.4200, were determined. The accuracy was also determined for AD measurements using the formulation of Garcia Mitacek et al. (2015), *i.e.*, DBB = (AD in mm – 35.1042)/0.7060.

Newborn kitten biometric measurements

Postpartum HD, CRL and body weight as well as gender were recorded for each kitten in the litter. If the queen needed medical/ surgical intervention for delivery, these measurements were obtained immediately after the neonates were clinically stable. If the cat gave birth at home, the measurements were performed within 24 hours after delivery. HD was measured with a caliper at the widest point of the skull, in front of the ears and behind the eyes (Figure 2). CRL was measured from the crown of the head to the bottom of the pelvis with a conventional measuring tape. Kittens were weighed on a digital kitchen scale.

Blood sample collection for progesterone measurements

Blood was taken from the queens into a serum or heparin tube at the time of inclusion into the study. In a few cases, blood collection was repeated as the cats did not give birth yet. Because most queens were presented due to dystocia in variable clinical condition, the amount of blood taken was kept minimum and therefore not always available for additional hormone assay. In three queens, progesterone was measured within two hours of collection with chemiluminescence (Immulite 2000, Siemens), which has been validated and reported for use in cats (Rohlertz, Strom Holst, & Axner, 2012). Serum/plasma collected from the other animals (N=17) was kept at -80ºC until analysis with an in-house radioimmunoassay as previously described (Goericke-Pesch et al., 2013; Goericke-Pesch, Georgiev, Atanasov, & Wehrend, 2013). Intra- and inter-assay coefficients of variation were 14.0% and 15.0%, respectively, and the limit of detection was 0.1 ng/mL.

Statistical analysis

Linear regression was used for the analysis of fetal ultrasonographic measurements (*i.e.,* BPD, AD, EDL and EDW) and DBB, and to compare BPD on the day of parturition with HD after birth. The average of each of the specific fetal measurements per litter was used for the calculations. The relationship between newborn biometric measurements, *i.e.,* HD, CRL, weight, and litter size was analyzed with linear regression, in which kittens were included individually. Breed comparison of newborn measurements (Domestic Shorthair, Bengal and Main Coon) was carried out with one-way ANOVA and with the Kruskal-Wallis test. The association between P4 concentration and DBB was also determined by linear regression. Serum P4 in one queen was considered an outlier (25.9 ng/mL on the day of parturition) and excluded from the statistical evaluation. This Domestic Shorthair queen was presented 67 days

after the first mating, because fetal fluids were gone for more than two hours, but parturition did not continue. An emergency C-section was performed.

Data analysis was carried out with IBM® SPSS® Statistics for Windows, Version 22.0 (Armonk, NY, USA). Significance level was set at $P<0.05$. Values are presented as mean ± standard deviation and range (minimum-maximum values).

Results

Fetal ultrasonography measurements

There was a moderate association between BPD and DBB and litter size ($r^2=0.423$, $P=0.001$; Figure 3). While BPD was increasing with time closer to parturition, litter size was inversely related to BPD. The linear regression equation derived is BPD (cm) = 2.381 + (0.051*DBB) - (0.035*Litter size). There was a weak positive association between AD and DBB ($r^2=0.267$, $P=0.012$; Figure 3). Litter size had no influence on AD. Neither EDL nor EDW was related to DBB ($P \geq 0.152$; Figure 4) or to litter size ($P \geq 0.930$).

Fetal measurements by ultrasonography varied not only among litters (Figure 3 and 4), but also among littermates. The differences in BPD, AD, EDL and EDW between the highest and lowest measurement in the same litter were 0.11 ± 0.08 cm (range: 0 - 0.31 cm), 0.33 ± 0.28 cm (range: 0.03 - 0.92 cm), 0.1 ± 0.1 cm (range: 0 - 0.28 cm) and 0.09 ± 0.06 cm (range: 0 - 0.19 cm), respectively.

Accuracy of parturition date prediction by BPD and AD

The accuracy of parturition timing by applying the linear regression equation of Beccaglia et al. (2008) on the BPD measurements was 40% within one day, and 53% within two days of prediction in our study population. Applying the formulations of Garcia Mitacek et al. (2015)

for estimation of delivery date, the accuracy was 27% (±1 day) and 50% (±2 days) for BPD, and 17% (±1 day) and 35% (±2 days) for AD in our data set.

Comparison of in utero BPD to postpartum HD

Even though BPD is a well-known fetometric parameter significantly related with gestational age, we compared BPD (per litter) on the day of parturition to HD of the newborn kittens (per litter) immediately after birth, as a control for our ultrasonography measurements. We found a moderate association between BPD and HD (r^2=0.712, P<0.0001; Figure 5). BPD (N=17) was 2.26 ± 0.14 cm (range: 1.93-2.52 cm), and HD of the same litters (N=17) was 2.32 ± 0.18 cm (range: 1.93-2.58 cm).

Newborn kitten characteristics

Our study included 85 kittens, of which 70 were born healthy, 12 were stillborn, two were euthanized because of cleft palate, and one died two days after birth. Biometric measurements were available from 84 newborns, in which HD, CRL and body weight were 2.26 ± 0.2 cm, 13.0 ± 1.1 cm and 93.8 ± 21.3 g, respectively. In the three most numerous breeds, *i.e.*, Domestic Shorthair, Bengal and Main Coon, HD and body weight of the Domestic Shorthair kittens was higher compared to the other two breeds ($P \leq 0.005$), and they were also longer than Bengal kittens ($P = 0.040$). Results by breed are presented in Table 1. Apart from breed differences, we also found considerable variation between the smallest and largest kitten in the same litter in HD (0.19 ± 0.10 cm, range: 0.05 - 0.32 cm), CRL (1.1 ± 1.0 cm, range: 0 – 3.7 cm) and body weight (25.0 ± 14.9 g, range: 1.0 – 56.0 g). Body weight and HD showed a moderate association with each other ($r^2 = 0.717$, $P < 0.0001$; Figure 6). There was a low to moderate relationship between CRL and HD as well as between CRL and body weight (r^2 =

231 0.364, $P < 0.0001$ and $r^2 = 0.398$, $P < 0.0001$, respectively). Gender had no effect on any of the
232 measurements when the whole population was analyzed ($P \geq 0.422$). Male and female kittens
233 were also not different when compared within each of the three breeds, *i.e.,* Domestic
234 Shorthair, Bengal and Main Coon ($P \geq 0.168$). Litter size was inversely related to HD ($r^2 =$
235 0.233, $P < 0.0001$) and body weight ($r^2 = 0.130$, $P = 0.001$), but not to CRL.
236
237 ***Maternal blood progesterone***
238 Progesterone levels were decreasing over time (Figure 7) and reached 3.18 ± 1.68 ng/mL on
239 the day of parturition. In the linear regression analysis, P4 levels showed a significant
240 association with DBB ($r^2 = 0.625$, $P<0.0001$).
241
242 **Discussion**
243 Several studies in the cat used fetal ultrasonography measurements for parturition day
244 prediction during weeks 4-5 to 9 of gestation (Beccaglia et al., 2008; Beccaglia & Luvoni,
245 2012; Garcia Mitacek et al., 2015; Gatel et al., 2015; Zambelli, Castagnetti, Belluzzi, &
246 Paladini, 2004), but none of them focused on the immediate prepartum period. Only a limited
247 number of measurements are available within the last few days of pregnancy (Garcia Mitacek
248 et al., 2015; Gatel et al., 2015). Therefore, our study was designed to examine the accuracy of
249 parturition day prediction by fetal ultrasonography measurements of BPD and AD obtained in
250 the last five days of gestation, and to introduce ED as a possible new predictor of the time of
251 delivery in queens. The association between BPD, AD and DBB was moderate to low
252 ($r^2=0.423$ and $r^2=0.267$), respectively. The lack of a strong relationship between fetal
253 measurements and DBB was also reflected by the low accuracy of parturition timing when we
254 applied the regression equations of Beccaglia et al. (2008) and Garcia Mitacek et al. (2015) to

255 our data. In our queens, which were examined close to birth, the accuracy of BPD was 27-40%
256 (±1 day) and 50-53% (±2 days), which is lower than 44-51% (±1 day) and 65-70% (±2 days)
257 reported in week 9 of pregnancy by others (Beccaglia & Luvoni, 2012; Garcia Mitacek et al.,
258 2015). The accuracy of our AD measurements (17% within one day and 35% within two days)
259 was even lower than that of BPD, but similar to previous findings (24-35%) of Garcia Mitacek
260 et al. (2015). This decrease in the reliability of delivery day prediction in the end of gestation
261 may be explained, at least in part, by the individual growth rates of the fetal heads, which was
262 reflected by differences in fetal BPD as well as in newborn HD among litters and littermates.
263 Furthermore, Beccaglia & Luvoni (2006) showed that in dogs, the accuracy of parturition date
264 prediction decreased in case of smaller or larger than normal litter sizes. The average litter size
265 in cats was reported to be 4.6 ± 1.7 (Sparkes et al., 2006), 3.9 ± 1.8 (Gatel et al., 2015) or 3.7
266 (Povey, 1978), and also depended on the breed, e.g. litter size in Main Coon queens was 4.6
267 (Sparkes et al., 2006) and 3.6 (Gatel et al., 2011). The variability of litter size in our study,
268 which included three singleton pregnancies, three queens with only two kittens, and two Main
269 Coons with seven kittens, may have hindered our parturition date estimation less accurate. The
270 low agreement between AD measurements and parturition timing could perhaps be explained
271 by the changes in the size of the fetal abdomen due to pressure by the littermates in utero or by
272 the ultrasound probe.
273 We found that BPD on the day of parturition in 17 queens was 2.26 ± 0.14 cm and HD in 84
274 newborn kittens was 2.26 ± 0.2 cm, which is similar to the studies of Beccaglia et al. (2008)
275 and Garcia Mitacek et al. (2015) predicting BPD of 2.34 and 2.31 cm on the day of delivery,
276 respectively. While the queens in both of these studies (three Norwegian Forest, four
277 European Shorthair and 16 mixed-breed cats) were relatively young (1-4 and 1-1.5 years of
278 age) and of similar body weight (3-4.5 and 2-4 kg), our animals represented seven different

breeds and were a heterogeneous group regarding age (0.8 – 8.5 years) and pregnant body weight (3.1 – 7.4 kg). This resulted in considerable variations in BPD (1.93 – 2.52 cm) and HD (1.70 – 2.63 cm) among litters and among kittens. In contrast to our average 2.26 cm BPD and HD at the time of birth, Zambelli et al. (2004) reported BPD of 2.6 cm about six days before parturition in eight domestic queens of 2-5 years of age and 3-3.5 kg of body weight. In our study, all BPD measurements were below 2.6 cm on the day of parturition, and HD was close to 2.6 cm only in a few newborns. According to Zambelli et al. (2004), all our queens should have delivered about six days later than they actually did. These differences between studies underscore the difficulties to estimate delivery date in various populations of cats shortly before parturition.

Furthermore, because it was impossible to clearly assign each fetal BPD measurement individually to the corresponding HD measurement after birth, we compared the mean BPD of some of the fetuses to the mean HD of all kittens in the litter. This is a bias of the method and may explain the moderate (r^2=0.712) association between the two parameters. Furthermore, we have to consider that in utero and postpartum measurements may not have been obtained at the same point of the head, which may further decrease the agreement between BPD and HD. BPD was taken at the widest distance of the skull between the two parallel parietal bones, while HD measurements were carried out directly caudal to the newborn's eyes and also included subcutaneous tissue, skin and hair.

While fetal biometric eye parameters were suitable predictors of parturition in ponies (Turner et al., 2006), neither EDL nor EDW were related to DBB in our study. Therefore, none of them seem suitable for prediction of parturition in queens. It is known that in cats, the eye of the fetus becomes visible on day 35, and the lens after day 50 of gestation (Zambelli & Prati, 2006). In Beagle dogs, the fastest growth rate of the eyes (~4mm/day) was found between

303 days 44 and 51, after which it slowed to ~1mm/day until day 58 (Boroffka, 2005). If we
304 assume that in cats a decrease in growth rate of the eyes towards the end of pregnancy occurs
305 similarly to dogs, it could explain the lack of relationship between ED and days before
306 parturition in our study. Additionally, our population was a heterogeneous group of queens
307 showing considerable variations in fetal eye measurements (0.8-1.3 cm in EDL on the day of
308 delivery). This is in agreement with the study on pony mares, in which a significant variation
309 in the growth pattern of the eye length was also reported across animals (Turner et al., 2006).
310 Because cats, unlike horses, are polytocous, differences among littermates may also account
311 for the variability in ED measurements. Indeed, we detected a difference up to 2.8 mm in eye
312 measurements among littermates. Furthermore, in contrast to foals, kittens as well as puppies
313 are delivered immature, and their eyelids separate 5-14 days after birth (Peterson & Kutzler,
314 2011). The difference between altricial species like carnivores and precocial species like
315 ungulates may explain why ED is a less useful biometric parameter for prediction of
316 parturition in cats compared to ponies.
317 Body weights of the kittens in our study were 55-143g, which is similar to the newborn
318 weights of 65-165g in 24 purebred queens of 11 different breeds (Gatel et al., 2011), but less
319 variable that those (30-170g) reported in a large questionnaire-based study of Sparkes et al.
320 (2006). These differences are partly due to breed, e.g. Norwegian Forest, Main Coon and
321 Chartreux kittens had higher birth weights compared to other cat breeds (Gatel et al., 2011).
322 Newborn Main Coons in our study were lighter than Domestic Shorthair and similar to
323 Bengals, which is surprising, as Main Coons usually produce heavier offspring (Gatel et al.,
324 2011; Sparkes et al., 2006). However, litter size, which was shown here as well as by Gatel et
325 al. (2011) to have an inverse relationship with newborn weight, may explain the apparent
326 controversy. Main Coon litters in our study were the largest *i.e.*, two queens had seven, and

another one had five kittens, which may have contributed to the lower kitten body weights. We found a moderate linear relationship ($r^2 = 0.717$) between newborn weight and HD, which is in agreement with Gatel et al. (2011), who described a significant relationship between birth weight, fetal BPD, femur length and time before parturition. Nevertheless, in their study (Gatel et al., 2011), femur length was superior over BPD in predicting birth weight. Body weight and CRL in our study showed only low to moderate association ($r^2 = 0.398$) indicating that CRL is not a good predictor of kitten birth weight. We did not measure CRL by ultrasonography, because the fetuses move and their bodies curve in late pregnancy rendering CRL measurements rather difficult to obtain. However, in the study of Garcia Mitacek et al. (2015), fetal CRL measurements of 6.17 cm ~2 days before parturition were approximately only half the size of our newborn measurements (13.0 ± 1.1 cm).

We found a decrease in maternal blood P4 levels towards parturition similarly to previous studies (Garcia Mitacek et al., 2015; Verhage, Beamer, & Brenner, 1976). On the day of birth, mean P4 in our study was slightly higher (3.18 ng/mL) than that (2.2 ng/mL) reported by Schmidt et al. (1983), and comparable to the P4 concentration of 2.99 ng/mL detected one day before delivery by Garcia Mitacek et al. (2015). In cats, P4 drops to baseline only after parturition (Verhage, Beamer, & Brenner, 1976), thus we did not expect to see baseline levels. However, P4 concentrations differed among queens ranging between 1 - 6.8 ng/mL on the day of birth, and in one cat, it was exceptionally high with 25.9 ng/mL. This queen had an emergency C-section because of dystocia. The three kittens were viable and of adequate size, thus it was not a premature delivery, which could have explained the high P4 concentration. Schmidt et al. (1983) also reported high (11.4 ng/mL) serum P4 in a queen on the day of parturition, which decreased to 2.6 ng/mL one day postpartum. From the clinical point of

view, this variability in maternal blood P4 levels close to and at the time of parturition underpin its limited use as a sole and accurate indicator of delivery date in cats.

In conclusion, when measured in the last five days of pregnancy, fetal biparietal diameter determined by ultrasonography is related to days before birth, but has moderate accuracy for parturition day prediction. In the cat, the use of abdominal diameter measurements for parturition timing is discouraged due to its low accuracy. The fetal eye diameter measured shortly before birth is not suitable for estimation of delivery day, too. The use of a combination of fetal ultrasonography and maternal serum P4 rather than each as a sole predictor of parturition is recommended.

Conflict of interest

The authors declare that they do not have any financial and personal relationships with other people or organisations that could inappropriately bias or influence their work.

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425 **Figure captions**

426

427 **Figure 1 Ultrasonography image of the feline fetal eye (vitreous body)**

428 The cursors show eye diameter length (yellow line) and eye diameter width (green line)

429 measured perpendicular to each other. The line of the eye diameter width is dissecting the lens

430 (echogenic circle within the vitreous body) approximately in the middle.

431

432 **Figure 2 Measurement of head diameter with a caliper in a kitten after birth**

433

434 **Figure 3: Fetal biparietal and abdominal diameter in relation to days before birth in the**

435 **cat**

436 BPD: biparietal diameter, AD: abdominal diameter

437

438 **Figure 4: Fetal eye diameter length and width in relation to days before birth in the cat**

439 EDL: eye diameter length, EDW: eye diameter width

440

441 **Figure 5: Fetal biparietal diameter on the day of parturition in relation to newborn**

442 **kitten head diameter**

443 BPD: biparietal diameter, HD: head diameter

444

445 **Figure 6: Newborn kitten head diameter (HD) in relation to body weight**

446

447 **Figure 7: Maternal blood progesterone in relation to days before birth in the cat**

448 P4: progesterone; N=19

449 **Tables**

450

451 **Table 1: Newborn kitten biometric measurements (N=84) taken shortly after birth is**

452 **shown by breed**

453 The kittens were born from 9 Domestic Shorthair, 6 Bengal, 3 Maine Coon, 2 Birman, 2

454 Norwegian Forest Cat, 1 European Shorthair and 1 Egyptian Mau queens. In one Main Coon

455 queen, data on one stillborn kitten was not available. a,b: breeds with different superscripts in

456 the same column are significantly different